# A STUDY OF CHROMOSOME ENDS IN SALIVARY GLAND NUCLEI OF DROSOPHILA

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## Introduction

The exact nature of chromosome ends has remained an unsolved problem in the study of chromosome structure. That the end is a specialized part with a characterized behavior is shown by the fact that an intact chromosome end never enters into a permanent association with any other part of the chromsomes and that its absence causes a change in behavior of the chromosome of which it was a part. This is clearly shown by the behavior in maize of broken chromosomes (McClintock, 1938, 1939, 1941, and 1942). Fusion occurs between the raw ends [3] of the two sister halves of the broken chromosomes resulting in a bridge configuration which breaks at anaphase. This "breakage-fusion-bridge cycle" may continue throughout the endosperm tissues, but in the embryo and plant tissues the raw end becomes "healed" and thenceforth no longer shows any tendency toward fusion. In other words, it then behaves as a normal chromosome end regardless of the type of tissue. This shows that there is a difference between a chromosome with a missing end and one with a "healed" end. The nature of the "healing" (or returning to the normal state) is not understood. Additional evidence that chromosomes are dependent for normal behavior upon certain conditions existing at the end is suggested by the paucity of reports of terminal deficiencies. It would be expected that terminal deficiencies would be found with a frequency, the square of the frequency of intercalary deficiencies requiring two breaks. (A small intercalary deficiency may require only one break by an X-ray, Demerec and Fano, 1941.) Nevertheless, terminal deficiencies occur only rarely and are the exception rather than the rule (Kaufmann, 1939a). This would appear to mean either that they are eliminated (Bauer, Demerec, and Kaufmann, 1938), or that there is a tendency for reunion between the two parts of the chromosome. In either case it is true that chromosomes with missing tips are much more rare than chromosomes with an intercalary region missing. There are enough exceptions, however, to show that it is possible for a chromosome to lose its tip and, in some manner, to reform or heal an end which allows the chromosome to function normally (the

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[3] The term "raw end" is used throughout to denote the new and unhealed end of a tipless chromosome. It carries no connotation concerning the physical and chemical state of the end and is merely the counterpart of "normal" and "healed."

terminal deficiencies reported by Demerec and Hoover, 1936; and Sutton, 1940). Also terminal inversions (Kaufmann, 1936; Kikkawa, 1937 and 1938), although rare, show the ability of a raw end of the chromosome to heal and assume the role of a normal end. From all of these data we get the impression that the end of a chromosome is not so much a separate entity as the word telomere denotes (for a recent discussion, see Muller, 1941) but rather a very special state which can be achieved by other parts of the chromosome under certain conditions. The separation of bivalents at meiosis presents further evidence that the chromosome end is unique in its behavior. Upon complete terminalization of chiasmata the ends of the separating chromatids frequently hold together with an amazing tenacity (for discussion see Hughes-Schrader, 1943a and b). An association of homologous ends may even be achieved without previous terminalization of chiasmata (Schrader, 1940a and b; Hughes-Schrader, 1943a). Also the bouquet stage strongly indicates a special function of chromosome ends, as pointed out most recently by Hughes-Schrader (1943b) and Schrader (1944).

The purpose of the present research has been to select and analyze some phenomenon characteristic of chromosome ends in the hope that the analysis would lead to a more complete understanding of the nature of that part of the chromosome. The phenomenon selected for study was that of association of non-homologous ends. This phenomenon occurs both in salivary gland nuclei of Diptera and in meiotic nuclei of various forms. The present study is limited to the salivary gland chromosomes of two wild type strains of *D. melanogaster*( Oregon-R and Swedish-b).

In salivary gland preparations the distal ends of any of the five long chromosome arms may be found adhered together, usually two-by-two. This phenomenon of non-homologous association of ends has been noted by several workers and its analysis was undertaken by Hinton and Sparrow (1941) and Hinton and Atwood (1941 and 1942). They found that some of the possible combinations of chromosome ends occurred with a significantly higher frequency than did others. The particular combinations of ends which occur with a non-random frequency varied from strain to strain but were characteristic of any one strain.

The present report adds to these conclusions the results from three lines of investigations: one, the study of the physical nature of terminal adhesions as demonstrated by mechanical manipulation; two, the genetical nature of adhesions, and the factors affecting adhesions; and three, the effect on the adhesion pattern of one strain of subsituting by genetical manipulation a chromosome or chromosome end from another strain. Since each of these points is best dealt with individually, the discussion of the results is presented in connection with the particular data to which it is pertinent. Therefore, the final discussion is of only a general and summarizing nature.

## Micromanipulation of Terminal Adhesions

For the purpose of studying the physical nature of terminal adhesions a technique was devised whereby two chromosome arms associated by a terminal adhesion could be pulled apart. This was accomplished with the aid of a Chambers micromanipulator, working with partially fixed glands, as suggested by Buck (1942). The glands were dissected from the larvae freehand under a binocular

microscope into a drop of aceto-orcein. At once, before hardening from the fixative resulted, the glands were transferred to a coverslip. A "V" of vaseline on the coverslip prevented the aceto-orcein from flowing away from the glands. The corner of another coverslip was placed over the glands, between the two walls of vaseline, and used to press the glands into a thin layer. The coverslip used to smear the glands was then removed and the coverslip with the smeared gland was inverted and placed on top of a moist chamber on the stage of a microscope above the micromanipulator. The various groups of chromosomes were searched quickly until a terminal adhesion was located. The chromosomes involved were identified and the configuration was drawn, using the 44X objective and 15X oculars. The microneedles were then raised into position and inserted into the chromosomes involved in the adhesion, and pulled apart until the chromosome region between the two needles (including the two adhered ends) broke at some point. This usually involved a marked degree of stretching—from two to three times the original length of the segment. The resulting configuration was then sketched; the figure marked with an ink spot; and the coverslip removed and the tissue further stained with aceto-orcein. The coverslip was subsequently sealed with paraffin face downwards onto a slide. The chromosome configuration was then relocated and the results studied using an oil immersion objective.

Twenty-eight such preparations were successfully completed. In 16 of these, the adhered chromosomes, which were stretched between the microneedles, separated between the two adhered chromosome ends. It should here be emphasized that this did not take the form of a mere separation of temporarily joined chromosome ends, but rather that the chromosome ends were tenaciously held together during the stretching by inter-band-like material which broke only after extreme attenuation. A careful study of the bands on the previously joined chromosome ends failed to reveal a single case in which the ends had gained or lost a band (see Figure 1). The only noticeable effect upon the chromosome ends was, in a few cases, a slight attenuation of the end itself.

Of the remaining 12 preparations, 6 failed to break—the terminal adhesions remained intact even though the chromosomes had been pulled to about three times their original length. After release from the needles, the chromosomes resumed an approximation of their original size and showed no marked effect of the previous stretching. No measurements were made at this point, but the degree of retraction of the chromosomes upon release is probably of the same order as that described by Buck (1942).

Six cases showed intercalary breaks. None of these six breaks were close to the adhered tips, three of them being in the chromocenter and the others in approximately the middle of the chromosome arm (2L in region 28; 3L in 67; and 2R in 52).

Eight successful attempts to disentangle the chromosomes in unbroken nuclei of semi-fixed glands by pulling them free with the microneedles produced three types of configurations. The first type was one in which the chromosomes formed a "star-shaped" configuration with all the bases of the chromosomes joined together and all the tips free. Two such configurations occurred. The second type was a continuous chain. This involved two terminal adhesions (2R-3R; 3L-X) both of which held during the stretching at the expense of the paired bases of the arms.

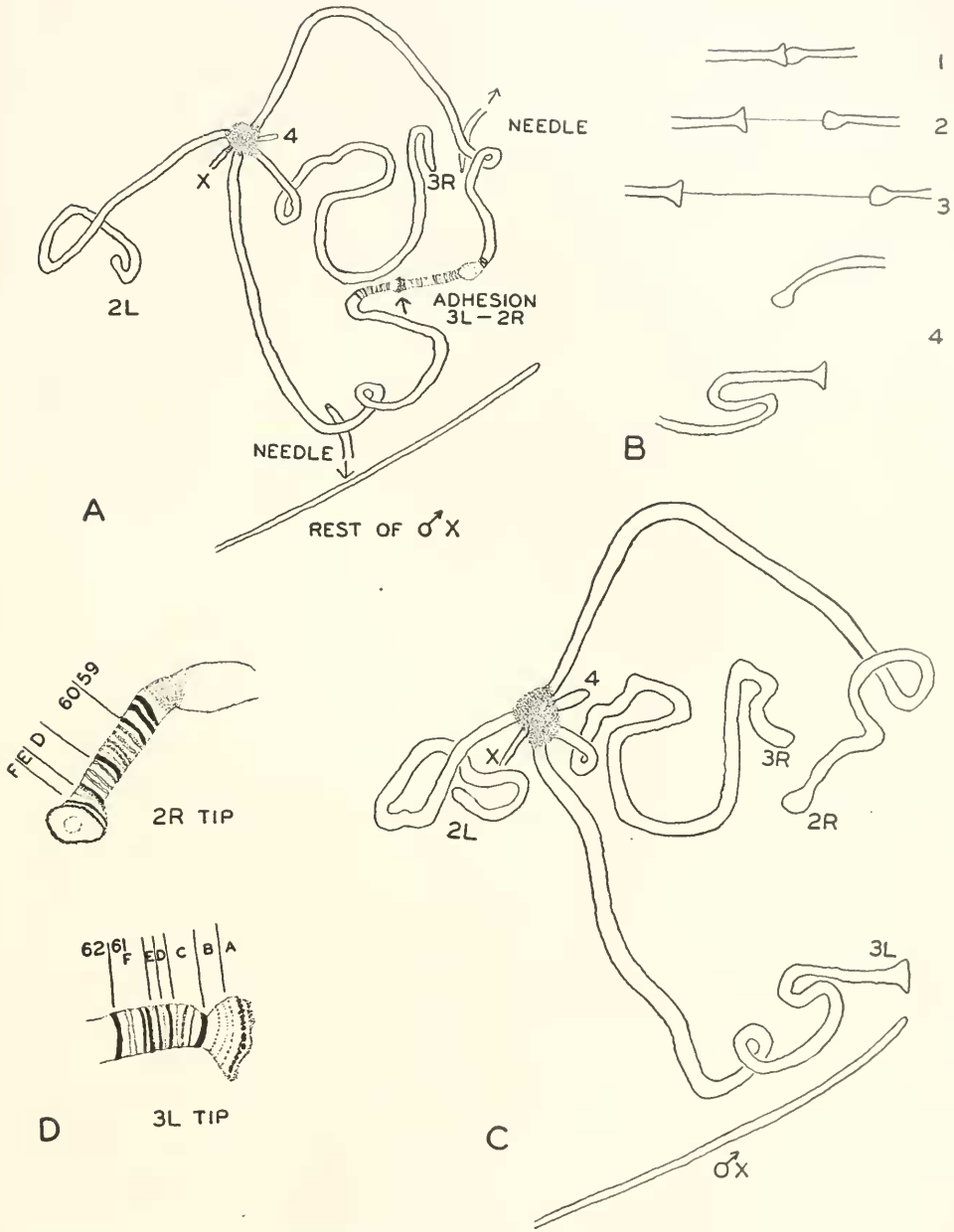


FIGURE 1. *A*. Configuration of chromosomes, sketched with needles of micromanipulator in position. *B*. Behavior of the two adhered ends while the needles were being moved apart. 1. Original position. 2. As the streching begins a thread-like material pulls out between the two ends. 3. The maximum extent of the stretching of the material between the two tips. 4. The thread-like material breaks and disappears leaving the ends free and relaxed. *C*. The same confirguration of chromosomes as in *A*, after the ends have broken apart. (Sketched using an oil immersion objective.) *D*. Detailed study of the bands at the ends of the two chromosomes which were previously adherent.

One such case was found. The third involved the chromosomes separating into two groups. This was due to the fact that the bases of the arms were originally joined in two groups—one autosome being alone. Two such cases were noted. Examples of this arrangement of the chromocenter were previously observed and recorded (but not published) when uninjured nuclei in whole mount preparations were being examined for other reasons (Hinton and Atwood, 1941 and 1942). These same arrangements and variations thereof were noted frequently in the broken nuclei. While pulling adhesions apart with the microneedles, the configuration of chromosomes would often be altered, stretching out into any one of the above mentioned types. At least five examples of each type are recorded.

No claim is made that the semi-fixed chromosomes used in these experiments accurately represent the living state. The work on these chromosomes does, however, throw light on such of their physical characteristics as are involved in the preparation of aceto-orcein slides, and shows the behavior of terminal adhesions and the chromosome arrangement from the beginning of fixation in the uninjured nucleus to the time the fully fixed and smeared preparation is observed.

The data show that a terminal adhesion is a condition in which the ends of the two chromosomes are strongly held together by a non-staining material which is continuous with the ends. They also show that the connection between the ends, even though tenacious, is still the weakest place along the chromosomes, with the exception of the adhered bases of the chromosomes. But the very fact that the ends invariably held together as firmly as they did, makes it unlikely that many cases of terminal adhesions are broken apart in the preparation of a slide.

Goldschmidt and Kodani (1942, 1943) noticed "the apparent strength of the cohesion which leads to a breaking off of tips which are attached to another chromosome in chromosome smear preparations." The foregoing data show that when two adhered chromosome ends are mechanically pulled apart, the phenomenon described by Goldschmidt and Kodani does not occur. Instead, it is evident that when a chromosome is fixed to the degree that is usual in preparing a smear, the adhered ends do not break apart with any ease. Even if they do break apart, it does not result in terminal deficiencies and translocations. In addition, if this type of aberration were produced by mechanical means, for every translocation there should be a deficient tip in the same nucleus, but Goldschmidt and Kodani fail to produce such evidence. It is possible that in a more thoroughly fixed chromosome, the phenomenon as postulated by Goldschmidt and Kodani could occur, but as shown elsewhere in the present paper, the frequency of terminal adhesions in a given stock at a given temperature does not vary, regardless of the length of time of fixation. If terminal adhesions break apart in smearing more readily after extreme fixation, then the frequency of terminal adhesions found on smear preparations should vary with the degree of fixation. It seems most unlikely from the data herein described that the small terminal deficiencies and translocations as seen by Goldschmidt and Kodani could be "pseudodeficiencies and translocations produced by mechanical breaks near the chromosome tip following cohesion of the telomere at the tips of two chromosomes." It seems much more likely that the deficiencies and translocations as seen by these authors were true deficiencies and translocations carried in the stocks which they examined; and that due to the small size of these rearrangements they failed to observe them in all nuclei, for it

would be extremely difficult to detect consistently an abberation as minute as one involving only one to four bands.

The idea that these small aberrations were carried as characteristics of their stocks does not seem unlikely in light of other work on the subject. Thus Kikkawa (1938) found a number of terminal structural differences between the chromosome ends of various *Drosophila ananassae*. His drawings of these extra bands on the chromosome ends are entirely similar to the drawings presented by Goldschmidt and Kodani (1943) for *Drosophila melanogaster*. Again, Dobzhansky and Dreyfus (1943) found, in Brazilian populations of *Drosophila ananassae*, "variations apparently identical with those described by Kikkawa." Also Bridges made a study of the free ends of the salivary chromosomes of *D. melanogaster* and found various examples of this type of abberation as characteristic of different strains. Unfortunately these data were never published in full but cases are listed in Drosophila Information Service No. 9—Df(2)Ore-R, Df(2)Sw-L, Df(2)Sw-R, Df(3)$D^3H^R$, Df(3)$Mz^L$. (See also Bridges and Brehme, 1944, page 59.) There seems, therefore, little doubt that the terminal abberations reported by Goldschmidt and Kodani, Kikkawa, Dobzhansky and Dreyfus, Bridges, and others, are all of the same nature and are inherent in the chromosomes of the individual stock being studied.

That these terminal deficiencies and translocations derive from the separation of terminal adhesions between non-homologous chromosomes, not in salivary gland nuclei, but during meiosis, remains a possibility. It is not inconceivable that a salivary gland nucleus is comparable to a pachytene nucleus of the first meiotic division. If a terminal adhesion occurred during the meiotic prophase and the ends later separated unequally, then the gamete receiving one of the involved chromatids would carry to the offspring a terminal deficiency or translocation. Such meiotic behavior represents one way in which terminal adhesions could play a role in the production of terminal aberrations. Terminal adhesions of non-homologous chromosome ends during the prophase of meiosis have been observed by Slack (1938) in Corixa, Schrader (1941) in *Edessa irrorata*, Ribbands (1941) in Habropogon, Rhoades and McClintock (verbal communication) in *Zea Mays*, and others (see Ribbands, 1941). Also terminal adhesions at meiosis may conceivably be involved with the deposition of matrix substance and the polarization of the chromosomes (see Schrader, 1941).

## The Genetical Nature of Adhesions and Factors Influencing Adhesions

### *Change of frequencies with time*

The frequency with which any two chromosome ends were associated was established for the Oregon-R strain in July 1940 (Hinton and Sparrow, 1941), and for the Swedish-b strain in July 1941 (Hinton and Atwood). These percentages are brought together in Table I for the sake of comparison. During the present investigation the frequencies in both Oregon-R and Swedish-b were reexamined (November 1943) by recording the types of adhesions observed from aceto-orcein smear preparations of glands. A marked difference is seen to exist when the current data are compared with those collected in 1940 and 1941. This difference is demonstrated in Table I. The data are from females only and are in per cents of the total cases examined in each case.

TABLE I

| Chromosome combinations | 1 Ore'R 7/40 | 2 Ore'R 11/7/43 | 3 Ore'R 11/17/43 | 4 Sw.-b 7/41 | 5 Sw.-b 11/28/43 | 6 Sw.-b 11/30/43 |
|---|---|---|---|---|---|---|
| X-2L | 17.3 | 21.0 | 20.0 | 14.0 | 3.0 | 1.0 |
| X-2R | 3.1 | 12.0 | 4.0 | 0.9 | 10.0 | 14.0 |
| X-3L | 17.3 | 5.0 | 8.0 | 16.8 | 8.0 | 0.0 |
| X-3R | 16.3 | 20.0 | 24.0 | 5.6 | 9.0 | 21.0 |
| 2L-2R | 3.1 | 2.0 | 4.0 | 9.3 | 1.0 | 2.0 |
| 2L-3L | 3.1 | 1.0 | 2.0 | 24.3 | 3.0 | 3.0 |
| 2L-3R | 5.1 | 1.0 | 1.0 | 10.0 | 1.0 | 1.0 |
| 2R-3L | 6.1 | 1.0 | 1.0 | 8.4 | 5.0 | 3.0 |
| 2R-3R | 16.3 | 31.0 | 30.0 | 2.8 | 52.0 | 50.0 |
| 3L-3R | 12.2 | 6.0 | 6.0 | 7.5 | 8.0 | 5.0 |
| Total cases | 98 | 100 | 100 | 107 | 100 | 100 |

It can be concluded from the data in Table I that:

1. The frequencies within each set of data are non-random. (Chi-square tests give probabilities of much less than .01 in each case that the data are similar to the expected random distributions.)

2. Oregon-R and Swedish-b in 1940–41 differed significantly from each other. (P—less than .01.)

3. Oregon-R, 1943, differs significantly from Swedish-b 1943. (P—less than .01.)

4. Oregon-R, 1940, differs significantly from Oregon-R 1943 (columns 2 and 3). (P—.01 to .02.)

5. Oregon-R, 11/9/43, does not differ significantly from Oregon-R, 11/17/43. (P—.70.)

6. Swedish-b, 1941, differs significantly from Swedish-b, 1943 (columns 5 and 6). (P—less than .01.)

7. Swedish-b, 11/28/43, does not differ significantly from Swedish-b, 11/30/43. (P—.05 to .10.)

The fact that both the Oregon-R and the Swedish-b patterns of adhesions have changed over a three year period of time suggests some mechanism for rapid evolution of the factors involved in terminal adhesions. The possibility exists that the stocks in question are not the identical stocks used in 1940–1941. However, since both Oregon-R and Swedish-b have changed, this seems less likely than if only one stock had changed.

*Dominance*

It was shown (Hinton and Atwood, 1941 and 1942) that when Swedish-b females were crossed to Oregon-R males, the $F_1$ females were identical with the Oregon-R parents in the relative frequencies of types of adhesions. This was interpreted to mean that the factors controlling the Oregon-R pattern of adhesions were in some way entirely dominant to the Swedish-b. Since, in the present investigation it has been shown that both the Oregon-R and Swedish-b pattern have

changed over a three year period, it was deemed advisable to reexamine the heterozygote (Ore'R/Sw.b). The results are presented in Table II.

The probability that the heterozygote has an adhesion pattern resembling that of the Swedish-b chromosomes is .10 as shown by an $\chi^2$ test. However, the probability that the heterozygote shows the same pattern as the Oregon-R parent is much greater (P—.50); yet, at the same time, an equal probability (.50) is shown that the heterozygote resembles the intermediate between the two parental patterns. Therefore it seems impossible at this time to conclude whether dominance has been maintained by Oregon-R or whether the dominance has been lost and Oregon-R and Swedish-b now have an equal ability to determine the adhesion pattern of the $F_1$, and either modify or compensate each other. However, if an examination is made of the only two classes wherein Oregon-R and Swedish-b are really dissimilar (X–2L and 2R–3R), then an $\chi^2$ test shows the following: the probability that Ore'R and Ore'R/Sw.-b are similar—.10; that Sw.-b and Ore'R/Sw.-b are similar—less than .01; and that Ore'R/Sw.-b and the theoretical intermediate between Ore'R and Sw.-b are similar—.70. It would appear from this analysis that Oregon-R has lost its dominance completely and that the heterozygote is merely the intermediate between the two parental strains in its adhesion pattern.

TABLE II

*Females, 1943*

| Chromosome combinations | Per cent adhesions Ore'R | Per cent adhesions Ore'R/Sw.-b | Per cent adhesions Sw.-b |
|---|---|---|---|
| X-2L | 20.5 | 12.5 | 2.0 |
| X-2R | 8.0 | 15.5 | 12.0 |
| X-3L | 6.5 | 6.5 | 4.0 |
| X-3R | 22.0 | 16.0 | 15.0 |
| 2L-2R | 3.0 | 3.0 | 1.5 |
| 2L-3L | 1.5 | 2.0 | 3.0 |
| 2L-3R | 1.0 | 2.0 | 1.0 |
| 2R-3L | 1.0 | 1.0 | 4.0 |
| 2R-3R | 30.5 | 39.0 | 51.0 |
| 3L-3R | 6.0 | 2.5 | 6.5 |
| Total cases | 200 | 200 | 200 |

*Cytoplasmic effect*

In order to observe the pattern of adhesions of identical chromosomes in different cytoplasms, the reciprocal crosses between Oregon-R and Swedish-b were made. Swedish-b females crossed to Oregon-R males produce $F_1$ daughters heterozygous for a complete set of Oregon-R chromosomes and a set of Swedish-b chromosomes, in Swedish-b cytoplasm (Ore'R/Sw.-b). Swedish-b males crossed to Oregon-R females give identical results with the exception that the cytoplasm is Oregon-R. The data from these two types of heterozygous daughters are presented in Table III.

The data show that the cytoplasm probably has no effect on the frequency of types of adhesions. An $\chi^2$ test gives a probability of .10 that the two sets of

data are similar. But here again, if the two crucial classes (X–2L and 2R–3R) are examined, they are exactly similar in the two sets of data. Therefore the factors which may be responsible for the specificities shown in terminal adhesions appear to be characteristic of the chomosomes and not dependent upon the genotype of the cytoplasm. This is in agreement with the conclusion reached by Hinton and Atwood (1941).

*Temperature effect*

A preliminary experiment indicated that temperature has an effect on the total incidence of terminal adhesions. By counting the number of nuclei examined and the number of terminal adhesions found, it was discovered that if the culture bottles remained at 17° during the development of the larvae approximately 20 per cent of the nuclei contained a terminal adhesion; if the culture bottle remained at

TABLE III

| Chromosome combinations | Per cent adhesions Ore'R/Sw.-b in Ore'R cytoplasm | Per cent adhesions Ore'R/Sw.-b in Sw.-b cytoplasm |
|---|---|---|
| X-2L | 13 | 12 |
| X-2R | 18 | 13 |
| X-3L | 5 | 8 |
| X-3R | 19 | 13 |
| 2L-2R | 1 | 5 |
| 2L-3L | 0 | 4 |
| 2L-3R | 3 | 1 |
| 2R-3L | 1 | 1 |
| 2R-3R | 39 | 39 |
| 3L-3R | 1 | 4 |
| Total cases | 100 | 100 |

23°, only 13 per cent of the nuclei contained an adhesion. In order to verify this conclusion the experiment was repeated with an attempt to collect more exact data. Females were allowed to lay eggs for 24 hours in culture bottles placed at 17°, and the flies then discarded. A second set of culture bottles received the same treatment at 23°. The culture bottles remained at their respective temperatures until the larvae were ready to pupate, at which time salivary gland preparations were obtained. Fifty figures were examined and the per cent of figures containing adhesions was calculated. Five such sets of data were collected from the cultures at 17°, and three from the cultures at 23°. The average percentage of adhesions per 50 nuclei occurring at 17° differs significantly from that at 23°. At 17° the mean percentage was 20 with a variation of $\pm 6$ per cent (14–26 per cent); at 23°, $13 \pm 4$ (9–17 per cent). The conclusion seems justified that the frequency of terminal adhesions varies with temperature.

To determine during which period of larval development temperature has its effect, culture bottles were placed at 23° for 24, 48, 72, and 96 hours respectively, and then removed to 17° where they remained until the larvae were ready to pupate. It can be seen from Figure 2 that there is little effect from the tempera-

ture as long as the larvae spend no more than the first three days of their development at 23°. After that the percentage of adhesions rapidly decreases. There are two ways to interpret this decrease in the frequency of adhesions: either terminal adhesions are sensitive to temperature during the fourth day of larval development; or there is an accumulative effect of temperature requiring at least three days. Further experimentation will make it possible to select between these two possibilities, but at this time only these preliminary data on temperature effect have been completed.

It was suspected that some cytological phenomenon could be correlated with the temperature effect. Salivary gland preparations were obtained from second instar larvae (the stage reached at the end of the third day at 23°). This preliminary cytological investigation failed to reveal a clear picture of the chromosomes since

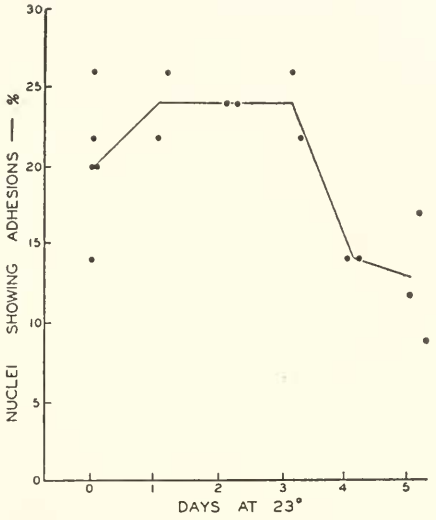


FIGURE 2.

they are most difficult to interpret at this early stage. It will be necessary to employ various cytological techniques before the question can be entirely settled. The results of the preliminary investigation do not exclude the possibility that terminal adhesions have not formed at the time of the second instar.

It will be noted, Figure 2, that the frequency of adhesions is slightly greater when the larvae are placed at 23° for the first several days and then returned to 17° than when the larvae undergo their entire development at 17°. If this is a significant difference, and only more detailed analysis will clarify that, then an explanation becomes difficult. It would indicate that there may be two occasions during the development of the chromosomes when temperature can effect terminal adhesion formation, or that there may be two different ways by which temperature exerts its effect.

In order to locate 100 terminal adhesions in the Swedish-b strain in cultures raised at 17° it was necessary to study, on the average, 45 smear preparations of pairs of salivary glands; but in order to locate 100 adhesions in the same strain

raised at 23°, it was necessary to study 128 smear preparations. Yet, when the 100 adhesions from 45 smear preparations are compared as to types of adhesions with the 100 taken from 128 preparations, it is apparent that the terminal adhesion pattern (specificities) remains unaltered. This is demonstrated by the data in Table IV. An $\chi^2$ test gives the following probabilities that these sets of data are similar: Column A compared to column C − P = .80; A to D = .80; A. to E = .95; B to C = .60; B to D = .05; B to E = .02; columns A plus B compared to C − P = .99; AB to D = .70; AB to E = .50; and columns A plus B compared to columns C plus D plus E − P = .70. Therefore, it can be concluded that temperature does not affect the types of adhesions which occur, but only the total incidence of adhesions.

TABLE IV

| Chromosome combinations | Per cent adhesions Sw.-b 17° | | Per cent adhesions Sw.-b 23° | | |
|---|---|---|---|---|---|
| | *A* | *B* | *C* | *D* | *E* |
| X-2L | 3 | 1 | 3 | 3 | 2 |
| X-2R | 10 | 14 | 14 | 7 | 8 |
| X-3L | 8 | 0 | 5 | 8 | 10 |
| X-3R | 9 | 21 | 17 | 13 | 8 |
| 2L-2R | 1 | 2 | 2 | 3 | 3 |
| 2L-3L | 3 | 3 | 2 | 2 | 2 |
| 2L-3R | 1 | 1 | 1 | 4 | 3 |
| 2R-3L | 5 | 3 | 5 | 5 | 7 |
| 2R-3R | 52 | 50 | 46 | 46 | 48 |
| 3L-3R | 8 | 5 | 5 | 9 | 9 |
| Total cases | 100 | 100 | 100 | 100 | 100 |

*Intercalary adhesions*

The ends of the chromosomes not only adhere to each other, but are also found adhered to regions along the length of the chromosome arms or to the bases of arms. Preliminary data on intercalary adhesions were reported by Hinton and Atwood (1941). Since that time 180 cases have been recorded which represent sufficient data to justify an analysis. All of these data were used to plot the frequency with which the various chromosome regions were involved in adhesions and this was compared with values expected on a random basis (number of adhesions divided by the number of chromosome regions as shown on Bridges map—100). A striking deviation from randomness was apparent, with some regions containing as high as 12 per cent of the total cases found, while in other places along the chromosomes as many as five consecutive regions contained no adhesions. In order to treat the data statistically in the most simple manner, each of the five long chromosome arms was divided into four parts by using every five regions on the Bridges map as a unit. These 20 sections were then compared with the theoretical random distribution. An $\chi^2$ test gave a probability of less than .01 that the adhesions could be distributed along the chromosomes at random. The conclusion is justified that there are certain regions of the chromosomes which are

associated in adhesions with a high frequency, while the majority of regions show the expected frequency.

The more distal regions of the chromosome arms show a higher frequency of adhesions than do the more proximal. This is best illustrated by Figure 3 in which are shown the five chromosome arms divided into four regions and plotted against the percentage of adhesions occurring per region. The data pictured in Figure 3 involve only intercalary regions, the data for the tips adhered to these regions being reserved for separate analysis.

Since the distal fourth of each chromosome arm shows a greater frequency of adhesions, it is necessary to assume, either that any material responsible for this type of pairing is present in greater abundance near the ends of the chromosome, or that due to the arrangement of the chromosomes, the distal regions are more accessible to the tips of other chromosomes. If the first hypothesis were true and it be postulated that this type of pairing is due to the attraction of heterochromatin, than a greater amount of intercalary heterochromatin should be located in the distal quarter of each chromosome arm than in any other region. There is no cytological evidence to support such an assumption, but since some heterochromatin regions present in dividing chromosomes do not appear in salivary gland chromosomes (Hinton, 1942) such a possibility is not eliminated. If the second hypothesis were true then the differential frequency would be due solely to mechanical causes.

Those regions which have a strikingly high occurrence of adhesions are shown in the following list:

| Chromosome arm | Regions |
|---|---|
| X | 2B, 3C |
| 2L | 21/22 |
| 2R | 56, 58 |
| 3L | — |
| 3R | 99/100, 100C/D |

Sixty-six additional places along the length of the chromosome were found to be involved in adhesions, but none of these with a surprisingly high frequency. Kaufmann (1939b) studied the position of X-ray induced breaks along the X chromosome and discovered at least 12 regions with a breakage frequency considerably higher than the expected frequency of a random distribution. He suggested that these regions contain heterochromatin which would account for the high breakage by X-rays and for the fact that these regions occasionally pair with each other and with the chromocenter. The question arises: Are the regions which show a high frequency of adhesions correlated with those showing a high breakage frequency? Table V, shown on the next page, makes such a comparison.

It is true that some regions with a high breakage frequency are found to be involved in adhesions, but on the whole neither a positive nor a negative correlation exists. Since those regions which have the highest adhesion frequency are not the same regions which show a high breakage frequency, non-specific heterochromatin certainly cannot be the important factor in both cases, although the possibility is not eliminated that it is important in one case or the other. Such a comparison as this is justified since the data indicate that neither the breakage frequency (Kaufmann, 1939b) nor the adhesion frequency (see following paragraph) varies for a given region in various strains.

Since it had been demonstrated that the pattern of end-to-end adhesions is different in different strains, the question arises as to whether the adhesion involving intercalary regions also express different specificities in different strains. It is difficult to answer this question definitely due to the relative paucity of data as compared to the several thousand places along the chromosomes that adhesions might occur, but in the Oregon-R strain 69 cases of intercalary adhesions have been recorded; and 46 in the Swedish-b strain, and when these cases are plotted against

TABLE V

| Regions found by Kaufmann to have a high breakage frequency | Regions found by Hinton to be involved in adhesions | |
|---|---|---|
| | Region | Number of adhesions |
| | 1C/D | 1 |
| 1F | | |
| | 2B | 11 |
| | 2D | 1 |
| | 3B | 1 |
| | 3C | 22 |
| | 3D | 1 |
| | 4B | 2 |
| 4E | | |
| | 5 | 3 |
| | 5/6 | 2 |
| 7B | 7B | 1 |
| 7C | | |
| 8B | | |
| | 8F | 1 |
| | 8/9 | 1 |
| | 9 | 2 |
| | 10A | 1 |
| 11A | 11A | 1 |
| 12D | 12D | 2 |
| 12E | | |
| | 14 | 1 |
| | 15/16 | 1 |
| 16E | 16E | 1 |
| 16F | | |
| | 18E/F | 1 |
| 19E | 19E | 2 |
| 19F | | |
| | 20B | 1 |

chromosome regions and the two sets compared it can be observed that the regions showing high frequency of adhesions are usually the same in each case and that the distribution is in general very similar. At least from the data on hand the indications are that even though intercalary adhesions are not at random, the specificity remains constant from strain to strain.

So far this discussion of intercalary adhesions has dealt only with the subterminal regions involved in the adhesion with no regard to which chromosome ends were involved. Data are presented in Table VI showing the number of times

each chromosome end is involved in an intercalary adhesion (in per cent of the number of cases observed).

From these data a difference in the behavior of the ends in the two strains is apparent. An $\chi^2$ test gives a probability of less than .01 that the two sets of data are similar. It therefore appears that the intercalary regions involved in adhesions remain the same from one strain to another but the chromosome ends involved vary from one strain to another. Another point of interest in these data is the correlation between the behavior of chromosome ends involved in intercalary ad-

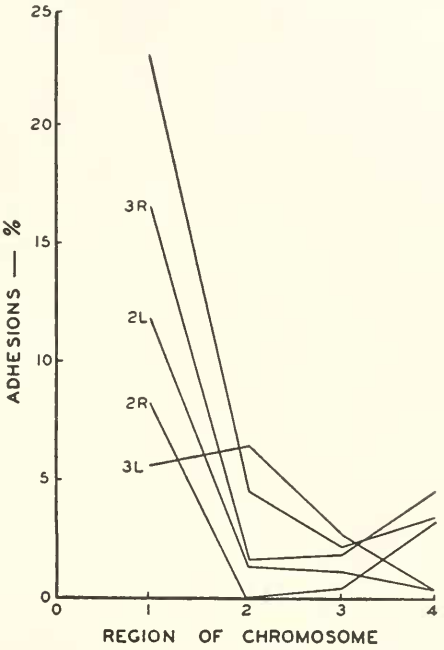


FIGURE 3. The number of intercalary adhesions is plotted against chromosome regions. Region 1 is the most distal in each case, but does not include the tip of the chromosome. Data for tips involved in intercalary adhesions are presented in Table VI.

hesions and those involved in terminal adhesions. It may be recalled that in Swedish-b, 2R and 3R adhere together with a frequency much greater than they do in Oregon-R. In Table VI it can be seen that both 2R and 3R are involved in intercalary adhesions with a frequency higher in Swedish-b than in Oregon-R. The same correlation exists for 2L and 3R. The only chromosome end which behaves the same in terminal adhesions in both strains is 3L, and it is found also to be involved in intercalary adhesions with about equal frequency in both strains (8 per cent and 6 per cent). A correlation is indicated between the potentialities of a chromosome end participating in terminal adhesions and in intercalary adhesions.

*Chromosome substitution*

The distinguishing feature of the adhesion pattern in the Oregon-R strain as contrasted with the Swedish-b lies in the considerably higher frequency of the X-2L

combination of chromosome ends (20.5 per cent as compared to 2.0 per cent) and lesser frequency of the 2R-3R combination (30.5 per cent as compared to 51.0 per cent). The eight other possible combinations of two chromosome ends occur with an approximately equal frequency in both strains. What will be the reaction of the Swedish-b X (which adheres to Sw.-b 2L in only 2 per cent of the adhesions observed) with an Oregon-R 2L that is accustomed to adhering to X in 20.5 per cent of the cases? The following experiment was designed to test the effect of a Sw.-b X chromosome upon the adhesion pattern of the Ore'R chromosome by substituting a Sw.-b X for an Ore'R X, leaving the autosomes entirely Ore'R. In order to achieve the substitution of one chromosome in a strain it is necessary genetically to mark each chromosome in such a manner that it can be identified and also to prohibit crossing over so that no alteration in the chromosomes can

TABLE VI

| Chromosome end | End involved in intercalary | | End involved with end | |
|---|---|---|---|---|
| | Strain | | Strain | |
| | Ore'R | Sw.-b | Ore'R | Sw.-b |
| X | 54 | 35 | 28 | 16 |
| 2L | 7 | 3 | 13 | 4 |
| 2R | 26 | 41 | 21 | 34 |
| 3L | 8 | 6 | 7 | 8 |
| 3R | 4 | 15 | 29 | 38 |

occur. If this is achieved, the result should be a strain of Drosophila entirely homozygous for Ore'R chromosomes with the exception of the X chromosomes which are homozygous Sw.-b. The obvious objection was foreseen, that regardless of the results of such a substitution no distinction could be made between the effect of the foreign chromosome as a whole and the effect of merely the foreign tip. It was therefore necessary to compare simultaneously a stock homozygous for Ore'R chromosomes with the exception of the tip of the X chromosome which would be homozygous Sw.-b. The creation of such a stock required crossing over at the tip of the X chromosome but under such conditions that the entire complement of chromosomes would remain Ore'R with the exception of this small terminal region. As a control to compare with these two altered stocks, it was necessary to recover from the crosses a stock carrying a complete homozygous set of Ore'R chromosomes. These three stocks which the experiment is designed to create may be pictured diagramatically as follows (the small fourth chromosome is ignored in these experiments):

| | X chromosome | | Chromosome 2 and 3 | |
|---|---|---|---|---|
| | Tip | Remainder | | |
| 1. | Ore'R | Ore'R | Ore'R | Ore'R |
| 2. | Sw.-b | Ore'R | Ore'R | Ore'R |
| 3. | Sw.-b | Sw.-b | Ore'R | Ore'R |

The genetical technique used to achieve these three stocks follows: In order to maintain the autosomes from one generation to another without losing their iden-

tity, chromosomes with dominant markers and inversions in both arms were carried heterozygously with the wild-type chromosomes (Cy $al^2$ $L^1$ $sp^2$ and Mé, In (3R)C, Sb e l (3) e). For ease in referring to these stocks, they are abbreviated in this paper as Cy L and Mé Sb, respectively. In order to obtain the proper crossover near the tip, an X chromosome marked with recessive mutants was used (sc ec cv $ct^6$ v $s^2$ f car $bb^1$ (abbreviated sc ec cv . . .). A crossover between scute (sc) and echinus (ec) would be in the 2 or 3 region of the salivary map or distal to 5.5 on the genetical map. This is relatively near the end of the X and would leave about 9/10th of the chromosome intact with only the distal 1/10th replaced. Other stocks used as markers were: y Hw d149 $m^2$ $g^4$ (abbreviated d149), Cl B, and Cy/Pm, $ds^{39k}$; H/C, Sb (abbreviated Cy/Pm; H/Sb). The series of crosses is summarized in Table VII.

TABLE VII

$P_1$ Sw.-b x sc ec cv . . . x Ore'R

$F_1$ $\frac{\text{sc ec cv . . .}}{\text{Sw.-b}}$ x sc ec cv . . . $\frac{\text{sc ec cv . . .}}{\text{Ore'R}}$ x $\frac{\text{Cy}}{\text{Pm}}$; $\frac{\text{H}}{\text{Sb}}$

$F_2$ $\frac{\text{Sw.-b} - \text{ec cv . . .}}{\text{sc ec cv . . .}}$ x sc − Ore'R; Pm; H

$F_3$ sc ec cv . . . x $\frac{\text{Sw.-b} - \text{ec cv . . .}}{\text{sc} - \text{Ore'R}}$; Pm; H x Ore'R

$F_4$ Ore'R x $\frac{\text{Sw.-b} - \text{Ore'R}}{\text{sc ec cv . . .}}$; Pm; H d149; Cy L; Mé Sb x Sw.-b − Ore'R; $\frac{\text{Pm}}{\text{Ore'R}}$; $\frac{\text{H}}{\text{Ore'R}}$

$F_5$ Sw.-b − Ore'R; $\frac{\text{Pm}}{\text{Ore'R}}$; $\frac{\text{H}}{\text{Ore'R}}$ x $\frac{\text{Sw.-b} - \text{Ore'R}}{\text{d149}}$; $\frac{\text{Cy L}}{\text{Ore'R}}$; $\frac{\text{Mé Sb}}{\text{Ore'R}}$

$F_6$ $\frac{\text{Sw.-b} - \text{Ore'R}}{\text{Sw.-b} - \text{Ore'R}}$; $\frac{\text{Ore'R}}{\text{Ore'R}}$; $\frac{\text{Ore'R}}{\text{Ore'R}}$

In order to establish a stock which has an entire Sw.-b X chromosome with Ore'R autosomes, and a control stock with a full complement of Ore'R chromosomes, the procedure diagrammed in Table VIII was used.

TABLE VIII

$P_1$ Sw.-b x d149; Cy L; Mé Sb d149; Pm; H x Ore'R

$F_1$ d149; $\frac{\text{Cy L}}{\text{Sw.-b}}$; $\frac{\text{Mé Sb}}{\text{Sw.-b}}$ x Ore'R $\frac{\text{d149}}{\text{Sw.-b}}$; $\frac{\text{Cy L}}{\text{Sw.-b}}$; $\frac{\text{Mé Sb}}{\text{Sw.-b}}$ x d149; $\frac{\text{Pm}}{\text{Ore'R}}$; $\frac{\text{H}}{\text{Ore'R}}$

$F_2$ $\frac{\text{d149}}{\text{Ore'R}}$; $\frac{\text{Cy L}}{\text{Ore'R}}$; $\frac{\text{Mé Sb}}{\text{Ore'R}}$ x Ore'R; $\frac{\text{Cy L}}{\text{Ore'R}}$; $\frac{\text{Mé Sb}}{\text{Ore'R}}$ $\frac{\text{d149}}{\text{Sw.-b}}$; $\frac{\text{Cy L}}{\text{Ore'R}}$; $\frac{\text{Mé Sb}}{\text{Ore'R}}$ x Sw.-b; $\frac{\text{Cy L}}{\text{Ore'R}}$; $\frac{\text{Mé Sb}}{\text{Ore'R}}$

$F_3$ $\frac{\text{Ore'R}}{\text{Ore'R}}$; $\frac{\text{Ore'R}}{\text{Ore'R}}$; $\frac{\text{Ore'R}}{\text{Ore'R}}$ $\frac{\text{Sw.-b}}{\text{Sw.-b}}$; $\frac{\text{Ore'R}}{\text{Ore'R}}$; $\frac{\text{Ore'R}}{\text{Ore'R}}$

After these three stocks were obtained, salivary smear preparations were made and 100 cases of terminal adhesions recorded from each. Table IX presents the data comparing the effect on the Ore'R pattern of terminal adhesions of a Sw.-b X chromosome versus a Sw.-b X chromosome tip versus an Ore'R X chromosome.

An examination of the data shows that the Sw.-b X (which seldom adheres with a Sw.-b 2L) when placed with an Ore'R 2L (which normally adheres frequently with Ore'R X) seldom adheres with it (column 2, Table IX). This introduced chromosome brings with it into the Ore'R strain the specificities which characterized it in the Sw.-b strain. Therefore the tendency of a chromosome to adhere or not to another chromosome is a property of the chromosome itself and not of the group of chromosomes as a whole.

But the experiment also makes possible a deeper analysis of chromosome behavior. When we are dealing with a chromosome complement of Ore'R in which only the tip of the X chromosome has been derived from the Sw.-b strain, there is still little adhesion between such an X and the left arm of chromosome 2 (column 3, Table IX). The strong attraction between the X-chromosome and 2L which is so typical of the Ore'R stock thus is abrogated by the substitution of a very small terminal portion of the X from the Sw.-b strain. It is therefore in the tip region

TABLE IX

| Chromosome combinations | 1<br>Ore'R X with Ore'R autosomes | 2<br>Sw.-b X with Ore'R autosomes | 3<br>Sw.-b tip on Ore'R X with Ore'R autosomes | 4<br>Sw.-b X with Sw.-b autosomes |
|---|---|---|---|---|
| X-2L | 17 | 1 | 1 | 1 |
| X-2R | 4 | 1 | 6 | 14 |
| X-3L | 10 | 7 | 5 | 0 |
| X-3R | 20 | 9 | 0 | 21 |
| 2L-2R | 2 | 5 | 3 | 2 |
| 2L-3L | 3 | 8 | 10 | 3 |
| 2L-3R | 3 | 2 | 5 | 1 |
| 2R-3L | 5 | 7 | 8 | 3 |
| 2R-3R | 30 | 36 | 39 | 50 |
| 3L-3R | 6 | 24 | 23 | 5 |
| Total cases | 100 | 100 | 100 | 100 |

of the chromosome that its adhesion properties are localized and the body of the chromosome as a whole is not responsible for the effect.

Finally it is to be noted that the X from Sw.-b (be it the whole X or only the tip replacing the Ore'R tip) fails to adhere frequently also to 3R of Ore'R. Since the X chromosomes of both stocks have a strong tendency to adhere to 3R if the latter is from the same stock, this must mean that the properties involved in the X-3R combination in one stock are different from those involved in the other stock.

Since two of the most frequent types of adhesions (X-2L; X-3R) are practically eliminated by introducing a Sw.-b X (or tip) into the Ore'R environment, it would be expected that the total frequency of adhesions should be reduced. This is not the case. The total number of adhesions per nucleus remains the same as in the parental strains (22 per cent of the nuclei contain adhesions in the Sw.-b tip stock; 20 per cent in the parental stocks). Thus the other eight possible combinations of chromosome ends (two-by-two) must rise in frequency to compensate for the two missing categories. Do they rise proportionally as might be expected? The answer is clearly that they do not rise proportionately. While some combinations

occur with an unaltered frequency, others are encountered much more often, and notably 3L-3R which soars from 6 per cent to 24 per cent. The indications seem to be that 3L and 3R, since they remain structurally unaltered, had the ability to adhere in the Ore'R strain but this was never realized due to a stronger attraction between X and 3R. In other words, once the stronger attraction is eliminated by bringing in a foreign X, the weaker attraction becomes the strongest remaining. (The word "attraction" is used throughout in a purely descriptive sense and in no way is an attempt made to imply physical forces.)

Thus, by substituting a foreign X chromosome and a foreign X chromosome tip into the Ore' R strain, the locus of specificity involved in terminal adhesion is found to be the end of the chromosome. The experiment also reveals unsuspected

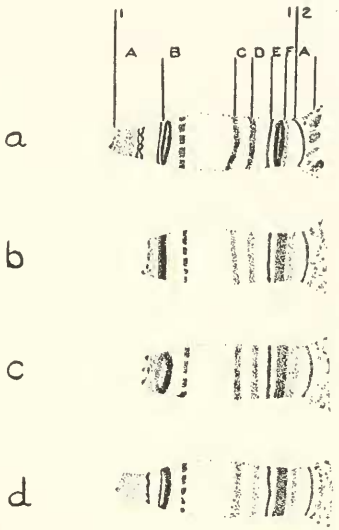


FIGURE 4. The distal end of the X chromosome in: *a*. Oregon-R. *b*. Swedish-b. *c*. Swedish-b on Oregon-R base. *d*. Marker stock.

potential attractions carried by the chromosome ends—attractions which result in adhesions only in the absence of stronger attractions.

*Cytological examination of the chromosome ends*

Detailed study was made of the banding at the end of the chromosome in the Sw.-b strain, the Ore'R strain, the Sw.-b tip on the Ore'R X base, and the marker stock used to obtain the crossovers (sc ec cv $ct^6$ v $s^2$ f car $bb^1$). This study demonstrated that the Ore'R and the marker stock X chromosomes are longer by a noticeable amount than either of the two Sw.-b X chromosomes. A study of Figure 4 shows that the Ore'R and marker X chromosomes have regions 1A 1-2-3 (Bridges map) while the others lack this. Whether this is a typical terminal deficiency as described in connection with the micromanipulation data in a previous section of the present paper, or not, the fact remains that there is a definite morphological difference in the X chromosome tips in these strains and furnishes

evidence in addition to the genetical evidence that the tip of the experimental stock is truly Sw.-b. It becomes obvious to suggest that the different specificities in terminal adhesion shown by the Ore'R and Sw.-b chromosomes may be due to the morphological difference. It was previously suggested (Hinton and Atwood, 1941) after a study of adhesions in a terminally deficient stock, that there are subterminal layers of properties which can operate if the tip is removed and the raw end heals. The new end has the ability to assume the role of the previous end, but not with the identical specificities.

## Discussion

Since all cells of an individual have the same descent one need not look to a difference between the chromosomes in order to explain the differences in adhesions among the cells of a single gland. Yet we also know from the experiments in the present paper that the end of the chromosome is the important factor in deciding the types of adhesions. This has meaning if we conceive of the tip as containing several properties for sticking, one of which, for example, involves a strong attraction for some other chromosome while another properly involves a weak attraction for still a different chromosome. If, at some stage in the development of the salivary gland, there is a movement of the chromosomes and an approximately equal chance that any chromosome end might pass close to any other chromosome end in the nucleus, it is logical that the two ends which have a stronger attraction for each other will show adhesion more often than will two ends with a weak mutual attraction which necessitates a closer approach before sticking can occur. This is suggesting that the different properties at the end of the chromosome responsible for the sticking have different sensitive volumes, or spheres of influence in which they are capable of attracting.

The tip replacement experiment furnishes the best clues as to the number of properties which must be assumed to be at the end of the chromosome. It is obvious from the data that all tips do not contain the same properties even though any two have some in common. Also, one end may exert an influence over another end so strong that weaker attractions are seldom realized except in the absence of the stronger attraction. Therefore the chromosome end must be assumed to contain properties seldom expressed due to the presence of more dominant properties. With these indications in mind it is possible to construct a model of the ends and the properties they contain. If the ends in Ore'R are designated as X-CA, 2L-CD, 2R-Bad, 3L-ac, 3-R-ABc; and if it be assumed that the letters used represent properties of the chromosome ends, and that the attraction between A and A is greater than between A and a which in turn is greater than between a and a, etc.; then the model expresses the various frequencies shown by the data.

Such a model is mainly a diagrammatic restatement of the data, but its main value is to indicate the amount of assumption necessary for a working hypothesis. According to this most simple model it is necessary to assume three or four properties per tip in order to include all data, and at least six different properties. One of these properties may be chromosome length, and another a general attraction between all parts of the chromosomes, but the rest of the properties must be assumed to be more specific in nature. These properties can be assumed to be either

TABLE X

(Assuming CC produced 20 per cent adhesion; Cc, 1 per cent; cc, 1; AA, 20; Aa, 6; aa, 1; BB, 20; dd, 3.)

| Combination | Attraction due to: | Frequency | |
|---|---|---|---|
| | | Expected | Actual |
| X-2L | CC | 20 | 20 |
| X-2R | Aa | 6 | 8 |
| X-3L | CC, Aa | 7 | 6 |
| X-3R | AA, Cc | 21 | 22 |
| 2L-2R | dd | 3 | 3 |
| 2L-3L | Cc | 1 | 1 |
| 2L-3R | Cc | 1 | 1 |
| 2R-3L | aa | 1 | 1 |
| 2R-3R | BB, Aa | 26 | 30 |
| 3L-3R | Aa, cc | 7 | 6 |

characteristics of a single substance or units of material at the end of the chromosome.

The idea that the whole phenomenon of terminal adhesions is due to a single heterochromatic attraction becomes impossible in light of the data presented in this paper. If quantitative differences in a single factor were responsible for the different frequencies of adhesions, as is assumed by the heterochromatic theory, then a mathematical model could be set up to fit. Such a model is impossible to formulate for one cannot explain in simple quantitative terms (with the adhesion frequencies following either the sum or the product of varying quantities) such frequencies of combinations of chromosome ends as have been shown to exist by the data. If heterochromatin is the substance involved in the formation of terminal adhesions, it becomes necessary to assume at least six types of heterochromatin either with stronger attractions between some types than between others or any one type possessing several properties. The same objection holds for the telomere theory. The telomere would have to consist of many parts each with different specificities. But regardless of whether we conceive of the end of the chromosome as being heterochromatins, compound telomere, or genic material, the indications are that it shows several specific attractions of various strengths, dependent upon proximity for expression, subject to change by mutation, and able to reform if removed or altered.

## SUMMARY

In order to obtain further insight into the nature of chromosome ends, the phenomenon of terminal adhesions in salivary gland chromosomes was studied.

Salivary gland chromosomes involved in terminal adhesions were stretched with the needles of a micromanipulator until the ends broke apart. No case was found in which the ends had gained or lost a band.

From determining which chromosomes were involved most frequently in adhesions, it is concluded that the frequency with which any two chromosome ends adhere together is non-random, differs in different strains, is subject to change

over a period of time, is not affected by the genotype of the cytoplasm nor temperature. However, the total incidence of adhesion is affected by temperature.

Intercalary regions associated with chromosome ends were found to be nonrandom.

A foreign chromosome end introduced into a strain demonstrates that the specificities shown in terminal adhesions are characteristics of the end itself, and reveals potential abilities of ends which are not realized under normal conditions.

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